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Authors: PLUMB, J. A., GRIZZLE, J. M., and DEFIGUEIREDO, J.

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## NECROSIS AND BACTERIAL INFECTION IN CHANNEL CATFISH (*Ictalurus punctatus*) FOLLOWING HYPOXIA<sup>1</sup>

J. A. PLUMB, J. M. GRIZZLE and J. DEFIGUEIREDO, Department of Fisheries and Allied Aquacultures, Agricultural Experiment Station, Auburn University, Auburn, Alabama, 36830, USA

**Abstract:** Apparently sterile lesions developed in the skin and musculature of channel catfish (*Ictalurus punctatus*) six days after fish were severely stressed by an oxygen depletion associated with a phytoplankton die-off in an 8.9 ha pond. Lesions were characterized by hemorrhage and necrosis. *Aeromonas hydrophila* was isolated from either the lesions or internal organs of all moribund fish necropsied the day after lesions were first found. As the water quality improved the health of the fish improved. Water quality data collected before and after the oxygen depletion, and associated fish mortality, are presented.

### INTRODUCTION

Bacterial diseases in fish generally do not develop simply as the result of exposing a host to an infectious agent.<sup>9</sup> In most instances, disease occurs as the result of complex interactions between pathogen, fish and environmental stress which affect the susceptibility of the host to diseases. Wedemeyer<sup>12</sup> and Snieszko<sup>10</sup> recently reviewed the role of stress in the susceptibility of fish to disease. Environmental stresses can affect the homeostatic mechanism of fish, thus reducing their resistance to pathogenic organisms.<sup>13</sup> Fish reared in intensive culture are exposed to extreme environmental fluctuations, and they may be more sensitive to stress than wild populations.

One of the most important environmental stresses on fish is low oxygen concentration.<sup>5</sup> Oxygen depletion in ponds, occasioned by "lake turn-over", phytoplankton die-off, or the result of feeding high nutrient diets leads to enormous biological and chemical oxygen demands<sup>1,11</sup> and is a common cause of death. Oxygen depletion may result in rapid and sudden die-offs; however, sub-acute oxygen depletion also may occur in ponds and relatively few fish will die as a direct result of depressed oxygen. The

role of chronic oxygen depletion as precursors to bacterial infection in fish has been discussed.<sup>7,10,12</sup>

*Aeromonas hydrophila* is a common water-borne bacterium which may be present in the tissues of apparently normal fish.<sup>4,6</sup> Whenever fish are exposed to environmental stress, or injury, it causes serious outbreaks of hemorrhagic disease with high mortalities.<sup>12</sup> The effect of low oxygen concentration on the prevalence of *A. hydrophila* infection in pond fish is well known but there is no documentation of the sequence of events before and after the O<sub>2</sub> depletion. Temperature, pH, high CO<sub>2</sub>, products of decomposition, and free ammonia in the water, all of which may interact in a pond where an O<sub>2</sub> depletion occurs, have a debilitating effect on fish.

During April and May, 1974 a culture pond with channel catfish (*Ictalurus punctatus*) at the Alabama Agricultural Experiment Station, Fisheries Research Unit, Auburn University underwent an acute oxygen depletion following a phytoplankton die-off in the pond. The limnological data collected before and after the oxygen depletion was reported by Boyd, Prather, and Parks.<sup>2</sup> The subsequent effect of the oxygen depletion on a disease condition in the fish population is described in this report.

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#### POND CONDITION AND FISH MORTALITY

The affected pond (S-1) is 8.9 ha with an average depth of 1.9 m. Source of water is surface runoff and a small stream. The water is soft, containing 15.6 mg/l total hardness and 25.2 mg/l total alkalinity.<sup>2</sup>

In January and February, 1973, S-1 was stocked with 66,000 (5,566 kg) channel catfish and 23,200 (35 kg) fathead minnows (*Pimephales promelas*). Early in 1974 an additional 5,000 kg of channel catfish were stocked in the pond to replace those removed by fishing. The fish were fed five days a week with a high protein diet and in late April, 1974 the standing crop was estimated at 3,470 kg/ha of channel catfish and 500 kg/ha of fathead minnows.<sup>2</sup>

A dense bloom of *Anabaena variabilis* was present in S-1. These phytoplankton suddenly died-off on April 29 and 30, 1974. Boyd, *et al.*<sup>2</sup> reported detailed limnological data on S-1 prior to and after the phytoplankton die-off. Before the die-off, dissolved oxygen in the surface water at 0700 hrs ranged from 6 to 9 mg/l, but on May 1 the oxygen level began to drop rapidly until on May 4 and 5 there was zero oxygen in the water column (Fig. 1). Morning water temperatures during the critical period ranged from 20.3 C on April 25, to 26.5 C on May 2. After May 6 the oxygen level began to improve and on May 8 was 9 mg/l at the surface. The pH of the water dropped from 9.4 on April 29 to a low of 6.7 on May 2 (Fig. 1).

Concentrations of ammonia nitrogen and carbon dioxide were very low before the phytoplankton die-off (Fig. 2). When the plankton decomposition began, ammonia nitrogen and CO<sub>2</sub> increased to 1.8 mg/l and 13 mg/l, respectively. As water quality improved and phytoplankton was reestablished the concentration of these compounds returned to pre-phytoplankton die-off levels.

Small channel catfish, weighing from 10 to 20 g each, showed signs of anoxia on May 2, the first day of acute oxygen

depletion (Fig. 1). On May 3 most fish in the pond, including catfish weighing 1 to 2 kg, were in distress. Agitators were installed in the pond and water from an adjacent pond was pumped into S-1; fish congregated at the source and the small amount of oxygen supplied by these methods allowed most of the population to survive. Dead fish were collected and weighed; the weight of dead channel catfish was 155 kg/ha and fathead minnows was 13.8 kg/ha. This comprised only 3.9% of the estimated standing crop.<sup>3</sup>

#### METHODS AND MATERIALS

Moribund channel catfish were collected and necropsied as follows: May 3, six fish; May 6, six fish; May 7, four fish and May 8, two fish. Moribund fish were not found on May 9, thus five channel catfish were seined from the pond for examination. On May 10, three fish were seined and examined. A daily search for moribund fish was made for two weeks after the last fish were collected.

Primary bacterial cultures were made on brain heart infusion agar plates.<sup>2</sup> Inocula were taken from livers or kidneys of all fish and from skin-muscle lesions when present. Bacterial isolates were identified as *A. hydrophila* according to the method of Bullock.<sup>3</sup> Wet mount preparations of scrapings from fins, skin, and gills of all fish were examined microscopically for possible parasite infestations.

Samples of liver, skin and muscle lesions, from the diseased fish and apparently normal skin and muscle tissues from the same fish were fixed in 10% phosphate buffered formalin and prepared for histological examination. Sections were stained with hematoxylin and eosin.

#### RESULTS AND DISCUSSION

Pathogenic bacteria were not isolated from the six moribund channel catfish necropsied on May 3, the second day fish were in distress. A significant level of parasites was absent. There were no

<sup>2</sup> Difco Laboratories, Detroit, Michigan.

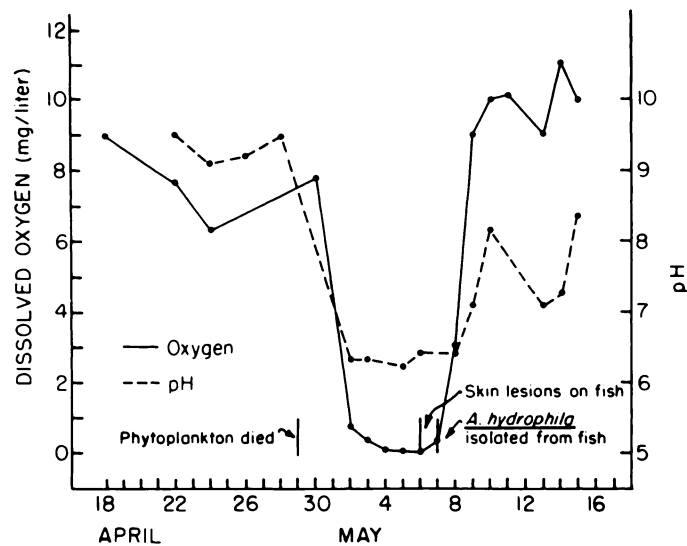


FIGURE 1. Oxygen concentration and pH in a channel catfish pond before and after a phytoplankton die-off which led to a fish kill and bacterial infection.<sup>2</sup>

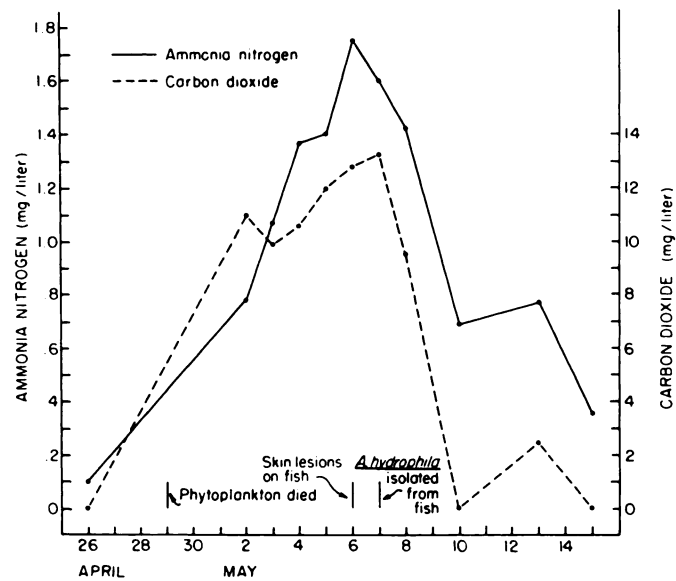


FIGURE 2. Ammonia nitrogen and carbon dioxide concentrations in a channel catfish pond before and after a phytoplankton die-off which led to a fish kill and bacterial infection.<sup>2</sup>

lesions on the skin or in the musculature at that time (Table 1). Lesions on the skin and in the musculature of six moribund catfish were observed on May 6. These lesions were characterized by light, depigmented areas of skin and petechiae (Fig. 3) which were very similar to the lesions caused by the enteric bacterium *Edwardsiella tarda*.<sup>1</sup> Bacteria were not isolated from the lesions or internal organs of these fish. Hemorrhage was pre-

sent in the dermis, hypodermis and in the underlying musculature; the striated muscle bundles were necrotic (Fig. 4).

Injury was not observed in the epidermis, dermis, hypodermis or superficial striated muscle of the apparently healthy tissues surrounding the lesions; however, the underlying striated muscle was necrotic, similar to the muscle in the lesions (Fig. 5). The livers were pale, had petechial hemorrhages, and the kidneys

TABLE 1. Necropsy of channel catfish from pond S-1 during and following an acute oxygen depletion.

Date of collection	Number necropsied	With skin lesions	With <i>A. hydrophila</i> infections	
			Muscle lesions	Kidney or liver
May 3 <sup>1</sup>	4	0	0	0
May 6	6	6	0	0
May 7	4	4	2	4
May 8	2	2	0	2
May 9	5	0	0	0
May 10	3	1	0	1 <sup>2</sup>

<sup>1</sup> Fish had been in distress for two days due to oxygen depletion.

<sup>2</sup> The fish with skin lesions had a bacteremia of *A. hydrophila*.

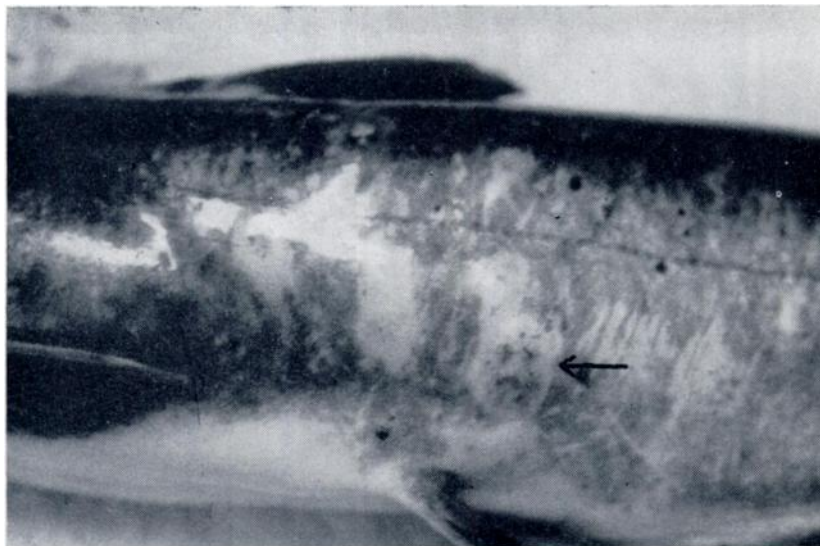


FIGURE 3. Channel catfish with hemorrhagic and necrotic lesion (arrow), and depigmented areas which developed 6 days after oxygen depletion. X 0.9.

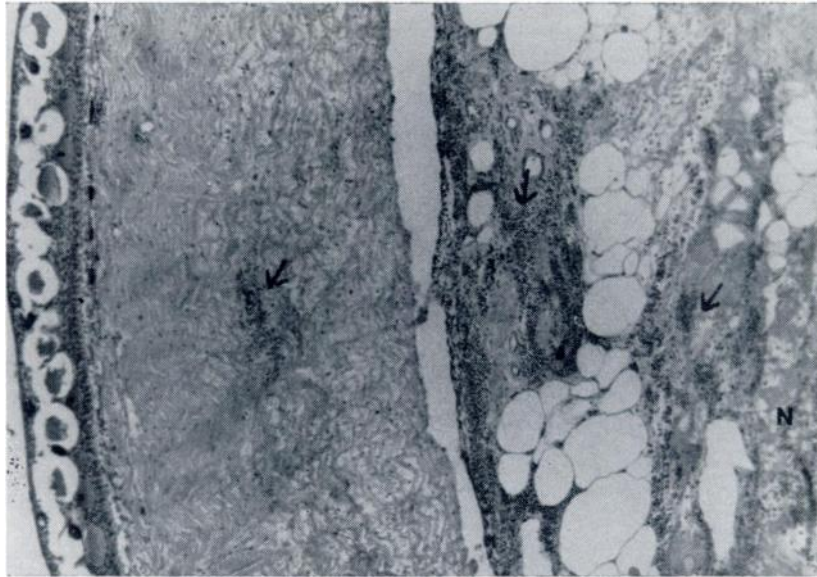


FIGURE 4. Section of skin and muscle with hemorrhage (arrows) in the dermis, hypodermis and necrotic muscle (N). Bacteria were not isolated from this lesion. H & E; X 90.

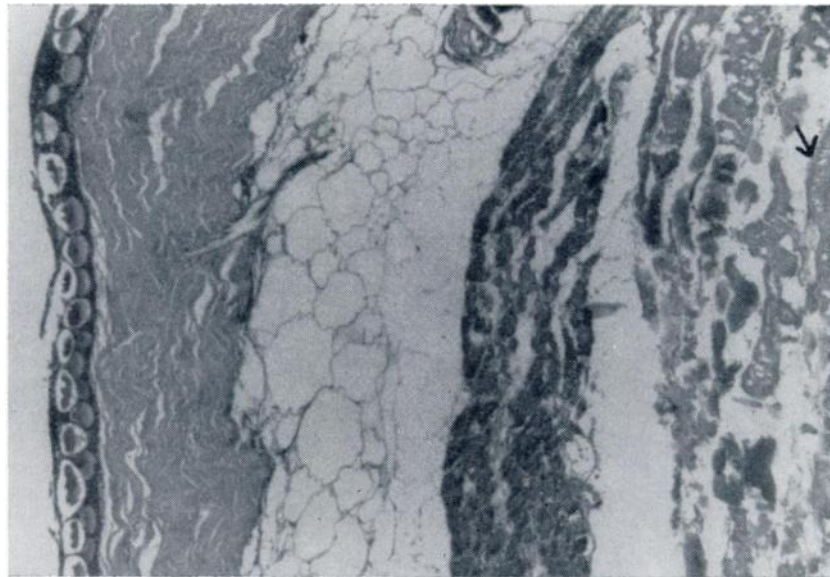


FIGURE 5. Skin and muscle from an area without a grossly apparent lesion. Underlying muscle is necrotic (arrow) and appears similar to the necrotic muscle in the lesion. H & E; X 73.

were slightly swollen. Intestines were hemorrhagic and intussusception had occurred.

The six fish necropsied on May 7 and 8 all had skin and muscle lesions similar to those observed on May 6. *A. hydrophila* was isolated from the lesions of two of six fish and from either the kidney or liver of all six. Only one of the eight fish seined and necropsied on May 9 and 10 had a bacterial infection; this isolate was not from a lesion. External parasites were not found on any of the fish examined. During the two weeks following the last collection no moribund fish were seen in the pond.

The primary cause of mortality in pond S-1 was oxygen depletion resulting from decomposition of phytoplankton. Remedial measures taken to alleviate the reduced oxygen content prevented a massive mortality.<sup>2</sup> These included the application of potassium permanganate and triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) to the pond along with installation of agitators and pumping fresh water into the pond. However, reduction of oxygen, increase in ammonia and CO<sub>2</sub>, and rapid drop in pH (Fig. 1 and 2) placed a severe stress on the fish population.<sup>2</sup> We hypothesized that during the 2 to 3 day period of unfavorable environmental conditions, hypoxia occurred in peripheral areas of the

musculature and internal organs of some fish. As a result of hypoxia, cells in these areas became necrotic. The first signs of this condition became evident on May 6, seven days after the phytoplankton die-off. The absence of bacteria in the skin and muscle lesions and in other parts of the body of moribund fish examined on May 6 substantiates the theory that bacteria were not the primary cause of the lesions. Also, necrosis in the deeper muscle layers of adjacent tissues suggest that death of these cells resulted from physiologic imbalance due to lack of oxygen and possible buildup of metabolites. However, by the following day (May 7) necrosis was to the point that the epithelial integrity could no longer prohibit establishment of *A. hydrophila* in the lesions of some fish, and body resistance was lowered to the point that systemic infections ensued. Apparently only a small number of fish were affected because by May 9 and 10 most fish had recovered.

The detailed limnological data documenting the water conditions, collected before and after the phytoplankton die-off in pond S-1,<sup>2</sup> are probably among the most comprehensive available. When these data are combined with the results of fish necropsy, they show a strong correlation between environmental stress and the ensuing bacterial infection.

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